

VI. Claims

1. An isolated homozygous stem cell (HS).
2. An isolated homozygous stem cell (HS) derived from human.
3. An isolated homozygous stem cell (HS) derived from non-human species.
4. The isolated homozygous stem cell of claim 3, wherein the non-human species is selected from the group consisting of mouse, hamster, dog, cat, rabbit, ferret, mink, guinea pig, hedgehogs, cattle, sheep, goat, llama, horse, deer, pig, monkey, and ape.
5. An isolated homozygous stem cell derived from a method comprising:
 - (a) producing a mitotically activated homozygous post-meiosis I diploid germ cell by: fusing two oocytes or two spermatids, preventing the extrusion of the second polar body during oogenesis, allowing the extrusion of the second polar body and spontaneous self-replication under appropriate conditions, or transferring two sperm or two haploid egg nuclei into an enucleated oocyte;
 - (b) culturing said activated homozygous post-meiosis I diploid germ cell to form a blastocyst-like mass; and,
 - (c) isolating homozygous stem cells from the inner cell mass of said blastocyst-like mass.
6. An isolated homozygous stem cell derived from the method of claim 5, further comprising screening stem cells that are homozygous by genotyping when a mitotically activated post-meiosis I diploid germ cell is produced by (a) fusing two oocytes or two spermatids, or (b) transferring two sperm or two haploid egg nuclei into an enucleated oocyte.
7. A method of producing homozygous stem cells comprising:

Cell I 1-6, 70-72, 73, 75, 80, 82, 83, 84
 meth II 7-8, 74, 76, 78, 79, 81, 85, 87, 88
 diff III 9-32, 33-43, 59, 77, 80
 IV 44-50, 62-69
 V 53-61

- (a) producing a mitotically activated homozygous post-meiosis I diploid germ cell by: fusing two oocytes or two spermatids, preventing the extrusion of the second polar body during oogenesis, allowing the extrusion of the second polar body and spontaneous self-replication under appropriate conditions, or transferring two sperm or two haploid egg nuclei into an enucleated oocyte;
- (b) culturing said activated homozygous post-meiosis I diploid germ cell to form a blastocyst-like mass; and,
- (c) isolating homozygous stem cells from the inner cell mass of said blastocyst-like mass.

- 8. The method of claim 7, further comprising screening stem cells that are homozygous by genotyping when a mitotically activated post-meiosis I diploid germ cell is produced by (a) fusing two oocytes or two spermatids, or (b) transferring two sperm or two haploid egg nuclei into an enucleated oocyte.
- 9. A method of making a desired progenitor cell, differentiated cell, group of differentiated cells, or tissue type comprising inducing isolated homozygous stem cells to differentiate under suitable conditions.
- 10. The method of claim 9, wherein differentiation is accomplished by the inclusion of a cell regulating factor, hormone or cytokine in the culture medium.
- 11. The method of claim 9, wherein the desired cell or group of cells is keratinizing epithelial cells.
- 12. The method of claim 9, wherein said keratinizing epithelial cells are selected from the group consisting of keratinocytes of the epidermis, basal cells of the epidermis, keratinocytes of the fingernails and/or toenails, basal cells of the nail bed, hair shaft cells, hair-root sheath cells, and hair matrix cells.

13. The method of claim 9, wherein the desired cell or group of cells is cells of wet stratified barrier epithelia.
14. The method of claim 9, wherein the desired cell or group of cells is epithelial cells specialized for exocrine secretion.
15. The method of claim 9, wherein the desired cell or group of cells is cells specialized for secretion of hormones.
16. The method of claim 9, wherein the desired cell or group of cells is epithelial absorptive cells of the gut, exocrine glands, or urogenital tract.
17. The method of claim 9, wherein the desired cell or group of cells is cells specialized for metabolism and storage.
18. The method of claim 9, wherein the desired cell or group of cells is epithelial cells serving as the lining the lung, gut, exocrine glands, or urogenital tract or as a barrier.
19. The method of claim 9, wherein the desired cell or group of cells is epithelial cells lining closed internal body cavities.
20. The method of claim 9, wherein the desired cell or group of cells is ciliated cells with propulsive function.
21. The method of claim 9, wherein the desired cell or group of cells is cells specialized for secretion of extracellular matrix.
22. The method of claim 9, wherein the desired cell or group of cells is contractile cells.

23. The method of claim 9, wherein the desired cell or group of cells is cells of the blood and immune system.
24. The method of claim 9, wherein the desired cell or group of cells is sensory transducers.
25. The method of claim 9, wherein the desired cell or group of cells is autonomic neurons.
26. The method of claim 9, wherein the desired cell or group of cells is supporting cells of sense organs and of peripheral neurons.
27. The method of claim 9, wherein the desired cell or group of cells is neurons or glial cells of central nervous system.
28. The method of claim 9, wherein the desired cell or group of cells is lens cells.
29. The method of claim 9, wherein the desired cell or group of cells is pigment cells.
30. The method of claim 9, wherein the desired cell or group of cells is germ cells.
31. The method of claim 9, wherein the desired cell or group of cells is nurse cells.
32. The method of claim 9, wherein the desired cell or group of cells is derived from one of the embryonic germ layers comprising the ectoderm, endoderm or mesoderm.
33. A method of producing progenitor cells, comprising:
- (a) producing a mitotically activated homozygous post-meiosis I diploid germ cell by: fusing two oocytes or two spermatids, preventing the extrusion of the second polar body during oogenesis, allowing the extrusion of the

second polar body and spontaneous self-replication under appropriate conditions, or transferring two sperm or two haploid egg nuclei into an enucleated oocyte;

- (b) culturing said activated homozygous post-meiosis I diploid germ cell to form a blastocyst-like mass;
- (c) isolating homozygous stem cells from the inner cell mass of said blastocyst-like mass; and,
- (d) inducing differentiation of said homozygous stem cells to produce progenitor cells.

34. The method of claim 33, further comprising screening stem cells that are homozygous by genotyping when a mitotically activated post-meiosis I diploid germ cell is produced by (a) fusing two oocytes or two spermatids, or (b) transferring two sperm or two haploid egg nuclei into an enucleated oocyte.

35. The method of claim 33, further comprising isolating said progenitor cells, and maintaining permanent progenitor cell lines.

36. The method of claim 33, wherein cells from said blastocyst-like mass are induced to differentiate in the absence of undifferentiated stem cells.

37. A method for producing genetically altered progenitor cells, comprising:

- (a) inserting, removing or modifying a desired gene in an HS cell to create a genetically altered HS cell; and,
- (b) inducing differentiation of said genetically altered HS cell.

38. A method for producing genetically altered progenitor cells, comprising:

- (a) inserting, removing or modifying a desired gene in a progenitor cell derived from an HS cell to create a genetically altered progenitor cell; and
- (b) culturing said genetically altered progenitor cell to grow genetically altered progenitor cells.

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47. A method of therapy comprising administering cells obtained using the methods of claim 9, 33, 37, or 38 to a patient in need of such therapy, wherein the patient is an animal species, and the cells are derived from human.

48. A method of therapy comprising administering cells obtained using the methods of claim 9, 33, 37, or 38 to a patient in need of such therapy, wherein the patient is human, and the cells are derived from human.

49. A method of therapy comprising administering cells obtained using the methods of claim 9, 33, 37, or 38 to a patient in need of such therapy, wherein the patient is an animal species, and the cells are derived animal species.

50. A method of therapy comprising administering cells obtained using the methods of claim 9, 33, 37, or 38 to a patient in need of such therapy, wherein the patient is human, and the cells are human.

51. The method of claim 50, wherein the cells derived from human are allogenic.

52. The method of claim 50, wherein the cells derived from human are isogenic

53. A progenitor cell derived from a method comprising:

- (a) producing a mitotically activated homozygous post-meiosis I diploid germ cell;
- (b) culturing said activated homozygous post-meiosis I diploid germ cell to form a blastocyst-like mass;
- (c) isolating homozygous stem cells from the inner cell mass of said blastocyst-like mass; and,
- (d) inducing differentiation of said homozygous stem cells to produce progenitor cells.

54. A progenitor cell derived from the method of claim 53, further comprising screening stem cells that are homozygous by genotyping when a mitotically activated post-meiosis I diploid germ cell is produced by (a) fusing two oocytes or two spermatids, or (b) transferring two sperm or two haploid egg nuclei into an enucleated oocyte.
55. The progenitor cell of claim 53, further comprising isolating and maintaining said progenitor cells as permanent cell lines.
56. The progenitor cell of claim 53, wherein cells from said blastocyst-like mass are induced to differentiate in the absence of undifferentiated stem cells.
57. The progenitor cell of claim 53, wherein said homozygous stem cells derived from said blastocyst-like mass are induced to differentiate in a flat adhesive environment.
58. The progenitor cell of claim 53, wherein said homozygous stem cells derived from said blastocyst-like mass are induced to differentiate in a 3D adhesive environment.
59. The progenitor cell of claim 53, wherein said homozygous stem cells derived from said blastocyst-like mass are induced to differentiate in a microgravity environment.
60. The progenitor cell of claim 53, wherein said homozygous stem cells derived from said blastocyst-like mass are induced to differentiate by generating stemplasms in immunodeficient mice.
61. The progenitor cell of claim 53, wherein said progenitor cells are capable of differentiating into only one of the three embryonic layers, the ectoderm, endoderm or mesoderm.

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62. A method of therapy comprising administering the progenitor cells of claim 53 to a patient in need of such therapy.
63. A method of therapy comprising administering progenitor cells of claim 53, to treat a disease or condition selected from the group consisting of Parkinson's, Huntington's, Alzheimer's, ALS, spinal cord defects or injuries, multiple sclerosis, muscular dystrophy, cystic fibrosis, liver disease, diabetes, heart disease, cartilage defects or injuries, burns, foot ulcers, vascular disease, urinary tract disease, AIDS and cancer.
64. A method of therapy comprising administering progenitor cells of claim 53 to a patient in need of such therapy, wherein the patient is human, and the differentiated cells are derived from animal species.
65. A method of therapy comprising administering progenitor cells of claim 53 to a patient in need of such therapy, wherein the patient is an animal species, and the differentiated cells are derived from human.
66. A method of therapy comprising administering progenitor cells of claim 53 to a patient in need of such therapy, wherein the patient is an animal species, and the cells are derived animal species.
67. A method of therapy comprising administering progenitor cells of claim 53 to a patient in need of such therapy wherein the patient is human, and the cells are human.
68. The method of claim 67, wherein the cells derived from human are allogenic.
69. The method of claim 67, wherein the cells derived from human are isogenic

70. An isolated homozygous stem cell derived from a method comprising:
- (a) producing a mitotically activated homozygous post-meiosis I diploid germ cell by: fusing two oocytes or two spermatids, preventing the extrusion of the second polar body during oogenesis, allowing the extrusion of the second polar body and spontaneous self-replication under appropriate conditions, or transferring two sperm or two haploid egg nuclei into an enucleated oocyte;
 - (b) culturing said activated homozygous post-meiosis I diploid germ cell to form a blastocyst-like mass being surrounded by zona pellucida;
 - (c) releasing said blastocyst-like mass from said zona pellucida using the method of assisted hatching; and,
 - (d) isolating homozygous stem cells from the inner cell mass of said blastocyst-like mass.
71. The isolated stem cell of claim 70, wherein the method of assisted hatching comprises, fixing said blastocyst-like mass in a micromanipulator having two arms such that one arm holds said blastocyst-like mass in a fixed position, and the other arm applies acidified tyrodes solution to the surface of the zona pellucida in an area equal to about one-eighth of the total surface of said zona pellucida so that the zona pellucida becomes weakened and the blastocyst-like mass is released.
72. An isolated homozygous stem cell derived from a method comprising:
- (a) producing a mitotically activated homozygous post-meiosis I diploid germ cell by: fusing two oocytes or two spermatids, preventing the extrusion of the second polar body during oogenesis, allowing the extrusion of the second polar body and spontaneous self-replication under appropriate conditions, or transferring two sperm or two haploid egg nuclei into an enucleated oocyte;
 - (b) culturing said activated homozygous post-meiosis I diploid germ cell to form a blastocyst-like mass being surrounded by zona pellucida;
 - (c) transferring said activated post-meiosis I diploid germ cell on day 2 post-activation to a mitomycin C treated feeder layer of mouse embryonic fibroblasts until a blastocyst-like mass is formed;

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Yan



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For: Isolated Homozygous Stem Cells,
Differentiated Cells Derived Therefrom, And
Materials And Methods For Making And Using
Same

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PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the above-identified application, Applicant(s) herewith respectfully requests the following amendment:

IN THE CLAIMS:

Please add the following new claims

73. (New) An isolated homozygous stem cell derived from a method comprising:
- (a) producing a mitotically activated homozygous post-meiosis I diploid germ cell by allowing the extrusion of the second polar body and spontaneous self-replication under appropriate conditions;
 - (b) culturing said activated homozygous post-meiosis I diploid germ cell to form a blastocyst-like mass; and,
 - (c) isolating homozygous stem cells from the inner cell mass of said blastocyst-like mass.

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74. (New) A method of producing homozygous stem cells comprising:
- (a) producing a mitotically activated homozygous post-meiosis I diploid germ cell by allowing the extrusion of the second polar body and spontaneous self-replication under appropriate conditions;
 - (b) culturing said activated homozygous post-meiosis I diploid germ cell to form a blastocyst-like mass; and,
 - (c) isolating homozygous stem cells from the inner cell mass of said blastocyst-like mass.
75. (New) An isolated homozygous stem cell derived from a method comprising:
- (a) isolating a homozygous post-meiosis I diploid germ cell, wherein said germ cell is an oocyte which matured to metaphase II *in vivo*;
 - (b) mitotically activating said metaphase II oocyte by allowing the extrusion of the second polar body and spontaneous self-replication under appropriate conditions;
 - (c) culturing said activated metaphase II oocyte to form a blastocyst-like mass; and,
 - (d) isolating homozygous stem cells from the inner cell mass of said blastocyst-like mass.
76. (New) A method of producing homozygous stem cells comprising:
- (a) isolating a homozygous post-meiosis I diploid germ cell, wherein said germ cell is an oocyte which matured to metaphase II *in vivo*;
 - (b) mitotically activating said metaphase II oocyte by allowing the extrusion of the second polar body and spontaneous self-replication under appropriate conditions;
 - (c) culturing said activated metaphase II oocyte to form a blastocyst-like mass; and,
 - (d) isolating homozygous stem cells from the inner cell mass of said blastocyst-like mass.

77. (New) A method of making a desired progenitor cell, differentiated cell, group of differentiated cells, or tissue type comprising inducing isolated homozygous stem cells created by the method of claim 73 or 75 to differentiate under suitable conditions.
78. (New) An isolated homozygous stem cell derived from a method comprising:
- (a) producing a mitotically activated homozygous post-meiosis I diploid germ cell by preventing the extrusion of the second polar body during oogenesis;
 - (b) culturing said activated homozygous post-meiosis I diploid germ cell to form a blastocyst-like mass; and,
 - (c) isolating homozygous stem cells from the inner cell mass of said blastocyst-like mass.
79. (New) A method of producing homozygous stem cells comprising:
- (a) producing a mitotically activated homozygous post-meiosis I diploid germ cell by preventing the extrusion of the second polar body during oogenesis;
 - (b) culturing said activated homozygous post-meiosis I diploid germ cell to form a blastocyst-like mass; and,
 - (c) isolating homozygous stem cells from the inner cell mass of said blastocyst-like mass.
80. (New) An isolated homozygous stem cell derived from a method comprising:
- (a) isolating a homozygous post-meiosis I diploid germ cell, wherein said germ cell is an oocyte which matured to metaphase II *in vivo*;
 - (b) mitotically activating said metaphase II oocyte by preventing the extrusion of the second polar body during oogenesis;
 - (c) culturing said activated metaphase II oocyte to form a blastocyst-like mass; and,

- (d) isolating homozygous stem cells from the inner cell mass of said blastocyst-like mass.
81. (New) A method of producing homozygous stem cells comprising:
- (a) isolating a homozygous post-meiosis I diploid germ cell, wherein said germ cell is an oocyte which matured to metaphase II *in vivo*;
 - (b) mitotically activating said metaphase II oocyte by preventing the extrusion of the second polar body during oogenesis;
 - (c) culturing said activated metaphase II oocyte to form a blastocyst-like mass; and,
 - (d) isolating homozygous stem cells from the inner cell mass of said blastocyst-like mass.
82. (New) An isolated homozygous stem cell derived from a method comprising:
- (a) producing a mitotically activated homozygous post-meiosis I diploid germ cell by: fusing two oocytes or two spermatids, preventing the extrusion of the second polar body during oogenesis, allowing the extrusion of the second polar body and spontaneous self-replication under appropriate conditions, or transferring two sperm or two haploid egg nuclei into an enucleated oocyte;
 - (b) culturing said activated homozygous post-meiosis I diploid germ cell to form a blastocyst-like mass;
 - (c) transplanting said blastocyst-like mass into an animal host to create a stemplasm; and,
 - (d) isolating homozygous stem cells from said stemplasm.
83. (New) An isolated homozygous stem cell derived from the method of claim 82, further comprising screening stem cells that are homozygous by genotyping when a mitotically activated post-meiosis I diploid germ cell is produced by (a) fusing two

oocytes or two spermatids, or (b) transferring two sperm or two haploid egg nuclei into an enucleated oocyte.

84. (New) An isolated homozygous stem cell derived from the method of claim 82, wherein said blastocyst-like mass is transplanted under the kidney capsule.
85. (New) A method of producing homozygous stem cells comprising:
- (a) producing a mitotically activated homozygous post-meiosis I diploid germ cell by: fusing two oocytes or two spermatids, preventing the extrusion of the second polar body during oogenesis, allowing the extrusion of the second polar body and spontaneous self-replication under appropriate conditions, or transferring two sperm or two haploid egg nuclei into an enucleated oocyte;
 - (b) culturing said activated homozygous post-meiosis I diploid germ cell to form a blastocyst-like mass;
 - (c) transplanting said blastocyst-like mass into an animal host to create a stemplasm; and,
 - (d) isolating homozygous stem cells from said stemplasm.
86. (New) The method of claim 85, further comprising screening stem cells that are homozygous by genotyping when a mitotically activated post-meiosis I diploid germ cell is produced by (a) fusing two oocytes or two spermatids, or (b) transferring two sperm or two haploid egg nuclei into an enucleated oocyte.
87. (New) The method of claim 85, wherein said blastocyst-like mass is transplanted under the kidney capsule.
88. (New) A method of making a desired progenitor cell, differentiated cell, group of differentiated cells, or tissue type comprising inducing isolated homozygous stem cells created by the method of claim 78, 80, 82, or 85 to differentiate under suitable conditions.

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REMARKS

It is respectfully requested that the Examiner enter this amendment prior to examining the application on its merits. The new claims added by this amendment are fully supported by the original specification, particularly Example 1. No new matter has been added.

Respectfully submitted,
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